

# *Traccatichthys punctulatus* sp. nov., a new species of stone loach (Pisces, Nemacheilidae) from Guangxi, southern China

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## Abstract

*Traccatichthys punctulatus* is here described from the Liu-Jiang flowing into the Xi-Jiang of the Pearl River basin at Jinxiu County, Guangxi Zhuang Autonomous Region, southern China. It is distinguished from all congeners by having a unique body coloration of a series of brown spots along the lateral line and dorsal-fin. Other diagnostic characters, not unique to this new species, are caudal-fin and lateral body colorations, and absence of pectoral-fin tuberculation. Its validity is further confirmed by its monophyly recovered in a molecular phylogenetic analysis based on mitochondrial COI and Cyt *b* gene sequences and species-level divergence between the new species and its closest relatives.

## Key Words

Genetic divergence, morphological differentiation, species description, taxonomy

## Introduction

The loach genus *Traccatichthys* was originally established by Freyhof and Serov (2001) to include two species: a Vietnamese species, *Nemacheilus taeniatus* Pellegrin & Chevey, 1936 (type species), and a Chinese species (*Micronemacheilus pulcher* Nichols & Pope, 1927). Subsequent taxonomic revisions showed that this morphology-based Chinese species actually comprises multiple distinct species. Prokofiev (2004) described specimens from Sanya, Hainan Island, as a species (*M. zispi*) distinct from *M. pulcher*. While both species were referred to *Traccatichthys*, a third Chinese species of the genus (*T. tuberculum*) was described from a coastal

river (Jian-Jiang) of Guangdong Province, southern China (Du et al. 2012). Outside China, *M. bacmeensis* (= *T. bacmeensis*) was described from northern Vietnam within the Red River basin by Hao and Binh in Nguyen (2005). Although Kottelat (2012, 2013) treated both *T. zispi* and *T. bacmeensis* as synonyms of *T. taeniatus*, Du et al. (2012) recognized *T. zispi* as valid while retaining *T. bacmeensis* as a synonym of *T. taeniatus*. Endruweit's (2014) examination of the topotypical specimens of *T. bacmeensis* confirmed that this species is valid. Currently, the genus is composed of five valid species distributed across Guangdong, Guangxi Zhuang Autonomous Region (hereinafter referred to as Guangxi), Guizhou, Fujian, and Hainan Island in China, Vietnam, and Laos

(Jin 1984; Kottelat 2001, 2013). Given that *Traccatichthys* species are a small-sized, with conserved morphology and intraspecific phenotypic variation, leading to considerable taxonomic confusion and disputed classification, the current taxonomy requires molecular scrutiny.

During our field surveys in 2005 and 2024, several specimens of *Traccatichthys* were collected from Jinxiu Yao Autonomous County, Guangxi Zhuang Autonomous Region, China. The collected specimens exhibited distinct morphological features that differed from previously described species within *Traccatichthys*. A combination of detailed morphological examinations and molecular phylogenetic analyses confirmed these specimens as a new species, which is described herein.

## Materials and methods

A small portion of the right pelvic fin was clipped for molecular analysis, with the remaining tissue preserved in vials containing 99.9% ethanol. Tissue samples from three individuals were used for mitochondrial cytochrome c oxidase subunit I (COI) and cytochrome b (Cyt *b*) gene sequencing. COI was amplified using the primers FISH F1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FISH R1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'), and the Cyt *b* gene was amplified using universal primers L14724 (5'-GACTTGAAAAACCACCGTTG-3') and H15915 (5'-CTCGATCTCCGGATTACAAGAC-3'). DNA was extracted using a Rapid Animal Genomic DNA Isolation Kit (Sangon Biotech (Shanghai) Co., Ltd., China) following the manufacturer's protocols. The polymerase chain reaction (PCR) was carried out in a total volume of 25 µL, including 12.5 µL of 2 × Taq mix, 1 µL of each forward and reverse primer, 1 µL of DNA template, and 9.5 µL of double distilled water (ddH<sub>2</sub>O). Sequencing was performed by Sangon Biotech (Shanghai) Co., Ltd. (China).

The sequencing results were manually checked, corrected, and assembled using SeqMan in the Lasergene v7.1.0 package (DNASTAR, Inc., Madison, WI, USA). The assembled sequences were aligned using the MUSCLE algorithm in MEGA v11 (Edgar 2004; Tamura et al. 2021), with redundant segments trimmed to obtain consistent sequences and aligned sequences concatenated to form a combined COI and Cyt *b* data matrix, totaling 1,712 base pairs. The genome sequencing data were submitted to GenBank (Accession: PQ814574–PQ814590 for COI, PQ818764–PQ818780 for Cyt *b*).

The complete mitochondrial genomes of 17 Nemacheilidae species and two botiid species were retrieved from GenBank to serve as outgroup taxa. The phylogenetic placement of *Traccatichthys punctulatus* sp. nov. was determined using maximum-likelihood (ML) and Bayesian inference (BI) analyses, implemented using the CIPRES Science Gateway (Miller et al. 2010). The ML tree was constructed using RAxML-HPC v8 (Stamatakis 2014), with 1,000 rapid bootstrapping iterations. The BI tree was

constructed using MrBayes in XSEDE v3.2.7a (Ronquist et al. 2012). Two parallel runs were performed, with four Markov chains starting from a random tree. The chains were run for five million generations and sampled every 100 generations, with the first 25% of sampled trees discarded as part of the burn-in phase. The remaining trees were used to create a consensus tree and estimate Bayesian posterior probabilities (BPPs). The phylogenetic trees were visualized and edited using FigTree v1.4.4 (Rambaut 2009). Uncorrected pairwise distances (1000 replicates) based on mitochondrial COI sequences were estimated using MEGA v11 (Tamura et al. 2021).

All counts and measurements followed Kottelat (1990). Specimens examined were stored in the Guangxi Normal University (GXNU), Guilin; the Kunming Natural History Museum of Zoology at the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences, Kunming; and the Museum of Aquatic Organisms at the Institute of Hydrobiology (IHB), Chinese Academy of Sciences, Wuhan.

## Results

### *Traccatichthys punctulatus* sp. nov.

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Fig. 1

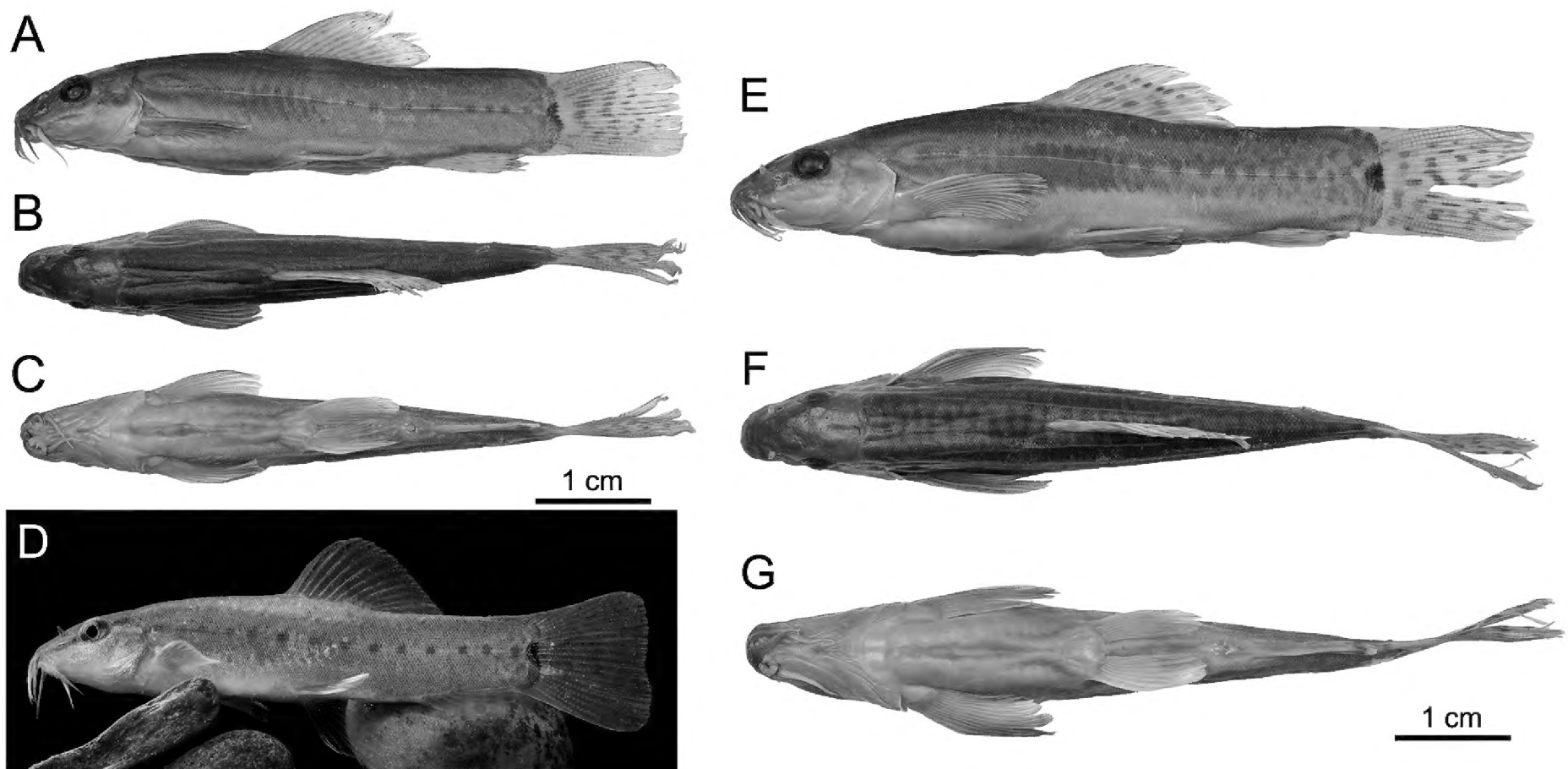
**Type material. Holotype.** • KIZ 2005013850, male, 47.4 mm standard length (SL); Dazhang River at Dazhang Township (23.8686°N, 109.8946°E), Jinxiu Yao Autonomous County, Laibin City, Guangxi, P. R. China; collected by J. Yang, Y.F. Huang, and D.P. Kong on October 23, 2005.

**Paratypes.** CHINA, Guangxi: Jinxiu Yao Autonomous County • KIZ 2005013849, 2005013860–63, five specimens, 40.1–63.7 mm SL, same as holotype • KIZ 2005009271–75, five specimens, 49.7–62.5 mm SL, from Gufan River at Toupai Town (24.3435°N, 110.1235°E), collected by D.P. Kong and J. Yang on October 18, 2005 • KIZ 2005013245–46, two specimens, 52.1–58.2 mm SL, Gumai River in Liuxiang Township (23.9595°N, 110.0332°E), collected by J. Yang, Y.F. Huang, and D.P. Kong on October 23, 2005.

**Other materials.** • DLN 20240034–36, preserved in 99% ethanol for molecular study, collected by J.H. Luo in Tongmu Town (24.1708°N, 109.9829°E), Jinxiu Yao Autonomous County, Laibin City, Guangxi, China, in April 2024.

**Diagnosis.** *Traccatichthys punctulatus* sp. nov. can be distinguished from all congeners by having many small spots organized in a single row along the mid-lateral line from the upper extremity of the gill opening to the caudal-fin base on the flank; a nearly-triangular black blotch on the caudal-fin base; lateral oblique brownish stripes confined only to the anterior half of body and more or less fused to constitute an oblong blotch not exceeding the adpressed tip of the pelvic-fin rays; no submarginal black streaks on the caudal-fin lobes; caudal fin with 3–4 rows of irregular spots.





**Figure 1.** Morphological characteristics of *Traccatichthys punctulatus* sp. nov. **A–C.** Lateral, dorsal, and ventral views of holotype KIZ 2005013850; **D.** Living photograph; **E–G.** Lateral, dorsal, and ventral views of morphologic variation KIZ 2005013862. Scale bars: 1 cm.

**Description.** Morphometric and meristic data given in Table 1. Body elongated, broad anteriorly and tapering posteriorly. Body deeper than wide, with maximum body depth at dorsal-fin origin being 17.7%–21.1% of SL, gradually declining from dorsal-fin origin to caudal-fin base. Snout blunt, slightly longer than lateral head length. Mouth arched and subterminal. Both lips papillated; upper lip bearing two rows of papillae, with two median papillae larger than lateral ones, and outer papillae slightly larger than inner papillae. Lower lip interrupted medially, forming wide V-shaped notch. Jaws covered by lips, with well-developed processus dentiformis on upper jaw. Nostrils closely set; anterior nostril elongated into tubular projection with pointed tip and posterior nostril oval. Eyes relatively large, with their diameter slightly smaller than interorbital width. Three pairs of barbels: inner rostral barbels reaching posterior nostril and outer rostral barbel extending to middle point of eye; maxillary barbel extending to vertical through posterior margin of eye.

Dorsal fin with three unbranched and 11–13 branched rays, with truncate distal margin; origin nearer to snout tip than to caudal-fin base, and inserted slightly before pelvic-fin insertion. Pectoral fin with one unbranched and 10–11 branched rays; inserted immediately anterior to vertical through posteriormost point of operculum; tip of adpressed fins reaching dorsal-fin origin. Pelvic fin with one unbranched and seven branched rays; inserted closer to anal-fin origin than to pectoral-fin insertion; tip of adpressed fins not reaching anus. Anal fin with three unbranched and five branched rays, originating equidistant to pelvic-fin insertion and caudal-fin base, or below or slightly anterior to vertical line of dorsal-fin tip; tip of adpressed fin not reaching caudal-fin base. Caudal fin slightly concave, with 16–17 branched

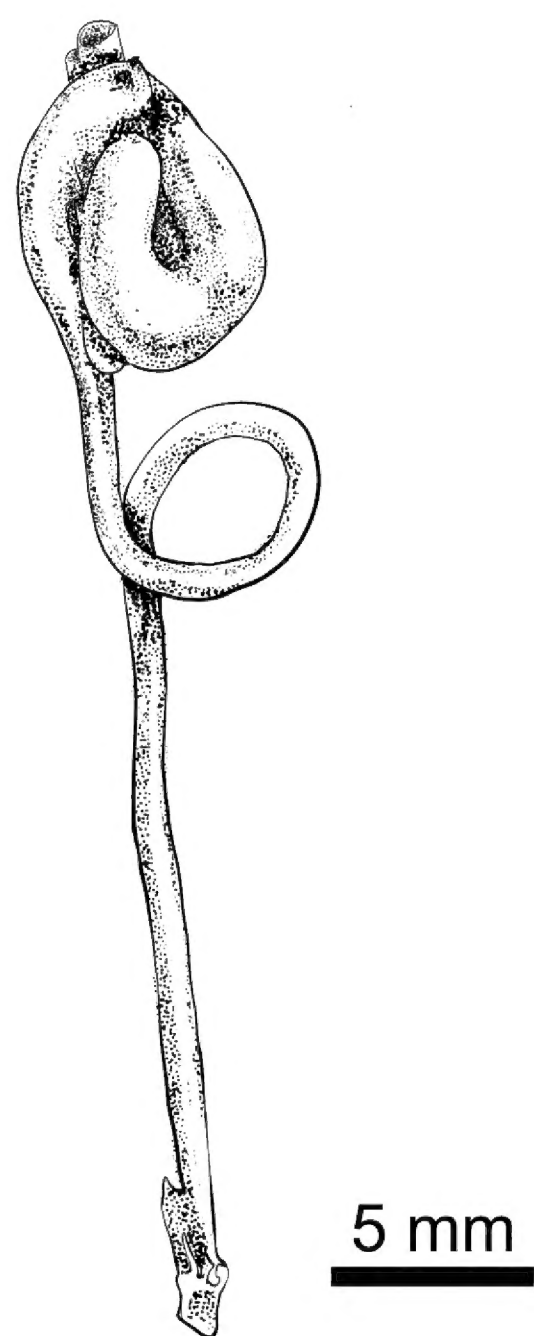
rays, slightly longer than deep (caudal-peduncle length/its depth 1.0–1.3). Irregular spots on dorsal, anal, and caudal fins; dorsal and anal fins with marginal stripes. Among the 12 examined specimens, individual variation in the dorsal surface (before the dorsal-fin) coloration was observed, with 10 specimens showing a plain, light gray back without markings and two displaying distinct patterning (Fig. 1E–G).

Except for head, body completely covered with small scales and embedded under skin. Lateral line complete, cephalic lateral system with 11–13+3 infraorbital canal pores, 7–8 supraorbital canal pores, 3 supratemporal canal pores, and 7–8 preoperculo-mandibular canal pores. Swim bladder bipartite; anterior chamber fully enclosed by capsule, and posterior chamber well-developed and ovoid, and connected to U-shaped stomach. Intestine short, looping once at midsection (Fig. 2). Gill opening large, extending downward beyond pectoral-fin insertion to ventral surface of head; first gill arch with 12–14 inner gill rakers and 0–2 outer gill rakers.

**Coloration.** In life, especially during breeding season, all fin rays pale pink, with lighter spots on dorsal and caudal fins (Fig. 1D). Many oblique bright green stripes along anterior lateral body and brightly silver spots on caudal-fin base. Distinct small round brown spots neatly arranged along mid-lateral line, gradually shallower from dorsal-fin base forwards. Back and sides light gray, abdomen pale yellow. Prominent blackish stripe from tip of snout through eyes to the occiput. In formalin specimens, dorsum brownish; flank and ventral side yellowish gray. Black stripe from snout tip through eye tending to blend with dark gray of head and dorsum. Fin rays faded, with black pigment deposits on dorsal surface of pectoral-fin rays, and spots on dorsal and caudal fins becoming more distinct.

**Table 1.** Morphometric measurements and meristic counts for *T. punctulatus* sp. nov.

Characters	Holotype	Paratypes (mean ± SD)
Total length (mm)	56.9	49.6–76.8 (64.7 ± 8.8)
Standard length (mm)	47.4	40.1–63.7 (53.4 ± 7.7)
<b>Percent of standard length (%)</b>		
Deepest body depth	20.7	17.6–21.1 (19.7 ± 1.1)
Head width	11.2	10.0–13.4 (11.9 ± 0.8)
Lateral head length	25.6	22.2–26.7 (24.9 ± 1.3)
Prepelvic length	53.3	50.6–58.9 (54.6 ± 2.1)
Preal length	75.9	68.9–80.7 (75.9 ± 2.7)
Prealus length	73.0	66.2–75.5 (71.6 ± 2.4)
Caudal-peduncle length	16.1	14.7–19.6 (17.0 ± 1.5)
Caudal-peduncle depth	14.7	13.2–15.6(14.4 ± 0.7)
<b>Percent of lateral head length (%)</b>		
Head width	43.7	39.6–54.6 (47.7 ± 4.3)
Head depth	52.8	55.4–64.9 (58.4 ± 2.7)
Eye diameter	23.5	21.1–27.8 (23.8 ± 2.5)
Interorbital width	28.9	22.3–36.0 (28.8 ± 3.8)
Snout length	41.7	42.8–51.1 (42.6 ± 2.6)
<b>Percent of caudal-peduncle length (%)</b>		
Caudal-peduncle depth	91.5	74.8–97.3 (84.8 ± 8.4)
Dorsal-fin rays	iii, 11	iii, 11–13
Pectoral-fin rays	i, 11	i, 10–11
Pelvic-fin rays	i, 7	i, 7
Anal-fin rays	ii, 5	ii, 5
Branched caudal-fin rays	16	16–17

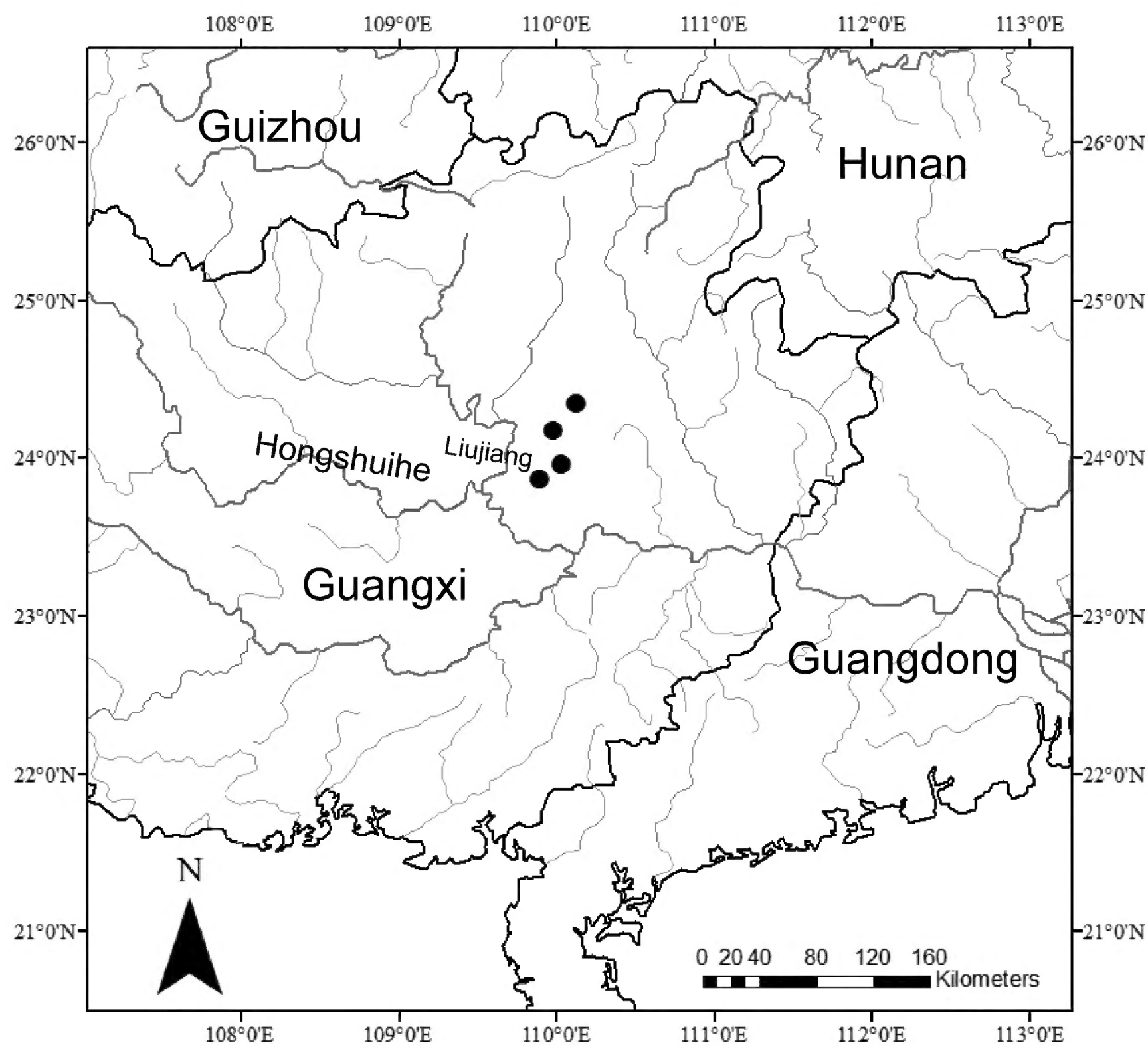


**Figure 2.** Stomach and intestine of *Traccatichthys punctulatus* sp. nov. based on morphological examination. Scale bars: 5 mm.

**Etymology.** The specific epithet *punctulatus* is derived from the Latin word “punctum”, meaning “spot” or “point”, referring to numerous spots observed on the dorsal and caudal fins and along the mid-lateral line of body. This body coloration represents a key unique diagnostic character used to differentiate it from all congeneric species. The common Chinese name suggested here for the new species is “斑点沙猫鳅” and the English name is “Spotted *Traccatichthys*.” The gender is masculine.

**Distribution.** The new species is so far found in streams on the western slope of Dayao mountains in Jinx- iu Yao Autonomous County, Laibin City, Guangxi, China, all of them discharging into the Liu-Jiang of the Zhu-Ji- ang basin (Fig. 3).

**Phylogenetic analysis and genetic divergence.** *Traccatichthys punctulatus* sp. nov. formed a separate lineage clustered within a well-supported clade (posterior probability = 1; bootstrap support = 100) alongside *T. pulcher*, *T. taeniatatus*, *T. tuberculum*, and *T. zispi*, in phylogenetic analyses using BI and ML based on the combined dataset of COI and Cyt *b* gene sequences (Fig. 4). Notably, *T. punctulatus* sp. nov. occupies a basal position within this clade of *Traccatichthys*, representing an early-diverging lineage in *Traccatichthys* and underscoring its distinct evolutionary history, which reinforces its status as a separate species. In addition, pairwise uncorrected *p*-distances between these species ranged from 1.58% to 10.79% for the COI gene (Table 2), with the maximum observed between *T. zispi* and *T. punctulatus* sp. nov. (10.79%).



**Figure 3.** Distribution map of *Traccatichthys punctulatus* sp. nov.

**Table 2.** Uncorrected *p*-distances between five species of *Traccatichthys* based on the mitochondrial COI gene.

Species	<i>T. pulcher</i>	<i>T. taeniatus</i>	<i>T. tuberculum</i>	<i>T. zispi</i>
<i>T. pulcher</i>				
<i>T. taeniatus</i>	0.0158			
<i>T. tuberculum</i>	0.0442	0.0410		
<i>T. zispi</i>	0.0558	0.0558	0.0416	
<i>T. punctulatus</i> sp. nov.	0.0915	0.0915	0.0852	0.1079

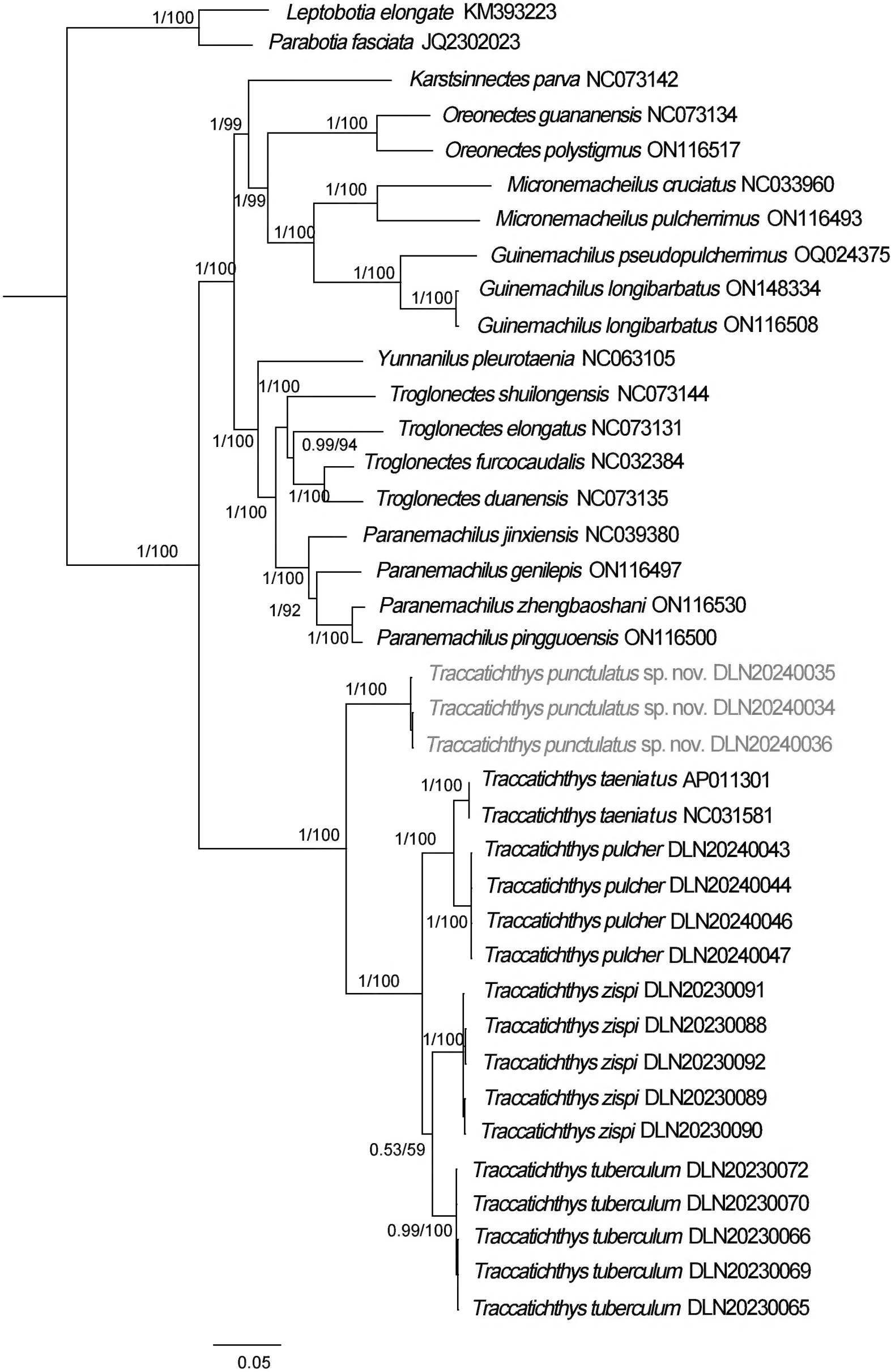
Discussion

The new species forms a distinct clade in the phylogenetic tree, with genetic distances from other congeners ranging between 8.52% and 10.79%. The clear genetic and morphological separation of *T. punctulatus* sp. nov. from its congeners strongly supports its recognition as a distinct species. Genetic analyses validated the observed morphological differences, particularly the unique spotted patterning on the dorsal and caudal fins as well as the lateral body. Morphologically, this new species shares typical characteristics

of *Traccatichthys*, including a complete lateral line, closely positioned anterior and posterior nostrils, papillated lips, and well-developed dentiform projections. However, the new species also possesses several unique traits that distinguish it from its congeners. Juveniles of *T. punctulatus* sp. nov. exhibit short, slightly separated lateral stripes that resemble those of *T. zispi*. By contrast, these lateral stripes in adults merge into serrated black blotches, with larger, irregularly sized spots along the lateral line and more prominent markings on the caudal peduncle. *T. punctulatus* sp. nov. exhibits irregular spots along the lateral midline extending from the operculum to the caudal-fin base, a character absent in other congeneric species. Additionally, the black blotch at the caudal-fin base is near-triangular, in contrast to the almost circular or rectangular spot seen in other species. These unique characteristics differentiate *T. punctulatus* sp. nov. from other species in the genus *Traccatichthys*.

In the dorsal fin, the unbranched rays are devoid of black pigmentation in the new species, distinguishing them from *T. bacmeensis* and *T. pulcher*, which possess such pigmentation. Instead of the marginal stripes observed in *T. bacmeensis*, *T. pulcher*, and *T. tuberculum*, the dorsal fin of *T. punctulatus* sp. nov. displays a row





**Figure 4.** Bayesian phylogram of *Traccatichthys* based on a combined dataset of mitochondrial COI and Cyt *b* sequences. Numbers on branches represent Bayesian posterior probabilities (BPPs) from BI analysis and bootstrap supports (BS) from ML analysis.

of irregular black spots. The anal fin exhibits a marginal black stripe, a characteristic absent in *T. bacmeensis* and *T. tubercul*. In contrast to *T. pulcher* and *T. tubercul*, the new species lacks marginal stripes along the upper

and lower edges of the caudal fin. Instead, the caudal fin is covered with numerous irregular black spots. In *T. taeniat* and *T. zispi*, however, all fins are unmarked and devoid of stripes. These pigmentation patterns collective-

ly distinguish *T. punctulatus* sp. nov. from its congeners. Additionally, individual variation was observed on the dorsal surface anterior to the dorsal fin, with some specimens exhibiting stripes while others lacked any markings. The source of this variation remains unclear and may be influenced by environmental factors, genetic diversity, or developmental stages, warranting further investigation.

In the phylogenetic analysis, *T. pulcher* and *T. taeniatus* exhibit a close genetic relationship with a relatively low genetic distance (1.58%); however, they show significant differences in external morphology. *T. taeniatus* is distinguished from *T. pulcher* by the absence of distinct black submarginal streaks on the dorsal and caudal-fin lobes, which are present in *T. pulcher*. Additionally, *T. pulcher* has larger oval blotches on the caudal peduncle and along the lower edges of the body from the ventral to the caudal, whereas such markings are absent in *T. taeniatus*. In terms of geographic distribution, *T. taeniatus* is primarily found in Vietnam and Laos, with its type locality in Phú Thọ Province, northeastern Vietnam. In contrast, *T. pulcher* has a broader distribution, with its type locality in Hainan, China, and occurs mainly in Guangxi, Guangdong, and Hainan, as well as in coastal cities of Vietnam from the Chinese border to central Vietnam. This clear geographic separation, combined with morphological differences, supports their status as distinct species despite their relatively low genetic divergence. However, further studies integrating additional molecular markers, population-level sampling, and ecological data are needed to fully resolve their taxonomic relationship.

Until now, the genus *Tracacanthys* comprises six recognized species, including the newly described species. Unfortunately, due to the lack of molecular materials, *T. bacmeensis* was excluded from our phylogenetic analysis, and the phylogenetic status of this species still remains unknown. No specimens of this species are available in this study. Morphological comparisons of the new species are made with *T. bacmeensis* in terms of Endruweit's (2014) redescription based on topotypic specimens.

There is controversy over the type locality of *T. pulcher* (Du et al. 2012). In terms of their original accounts, Nada Town and Sanya, Hainan Island, are the type localities for *T. pulcher* and *T. zispi*, respectively. However, the specimens we collected from Nada Town do not conform to *T. pulcher* (Figs 5, 6), but have no marginal and sub-

marginal bands on dorsal fin and upper and lower marginal bands on the caudal fin, thus conspecific with *T. zispi* (Fig. 7). In contrast, specimens we collected from Dongfang City and Baisha Li Autonomous County exhibit the characters typical for *T. pulcher*. Thus, we agree with Du et al. (2012)'s third explanation that the type locality of *T. pulcher* requires further investigation.

## Comparative material

*Taccatichthys pulcher*: KIZ 2002009935, 67.3 mm SL, Gongcheng, Guangxi; KIZ 2011001585–93, 39.5–75.1 mm SL, Rong'an, Liuzhou, Guangxi. F 000090–92, 59.0–69.6 mm SL, Dongfang City, Hainan Island.

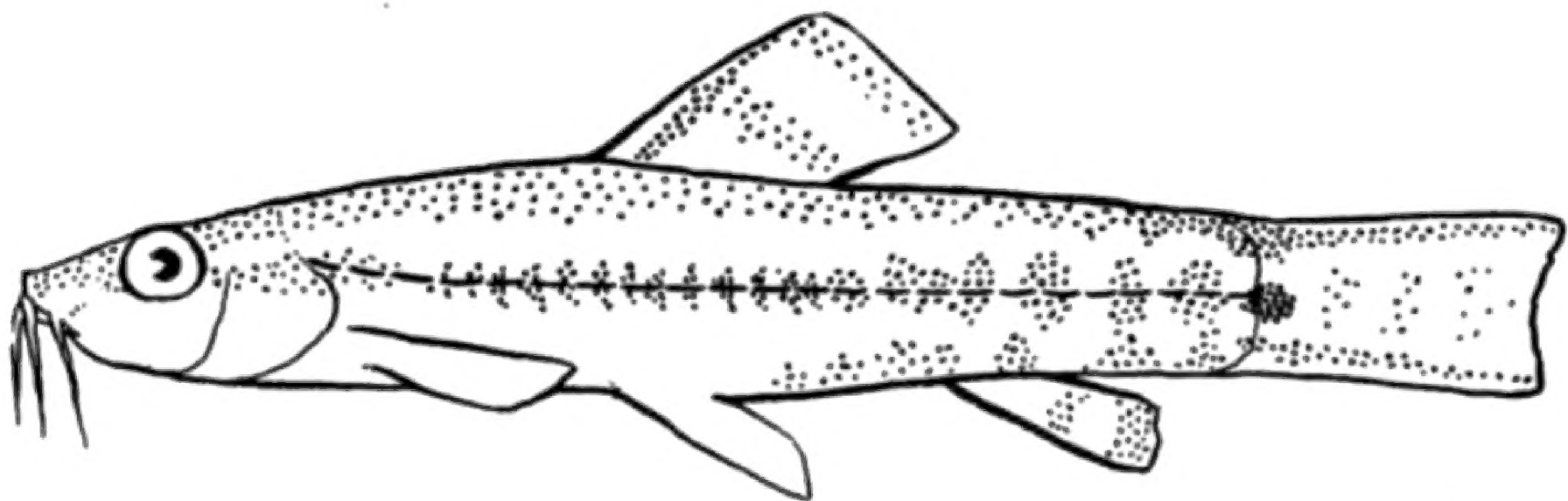
*Tracacanthys tuberculum*: IHB 201105029, 87.7 mm SL, IHB 201105004 201105007–18, 201105020, 201105022–8, 201105030, 54.5–75.1 mm SL, Jian-Jiang, Xinyi County, Guangdong, China.

*Tracacanthys zispi*: KIZ 2008006329–31, 55.8–68.2 mm SL, Hongshuihe, Hongshui Village, Wangxia Township, Changjiang County, Hainan; KIZ 200803457–3464 and KIZ 200803475–3482, 52.9–67.5 mm SL, Heilan Village, Yuanmen Township, Baisha County, Hainan. F 000059–65, 48.2–71.3 mm SL, Nada Town, Hainan Island.

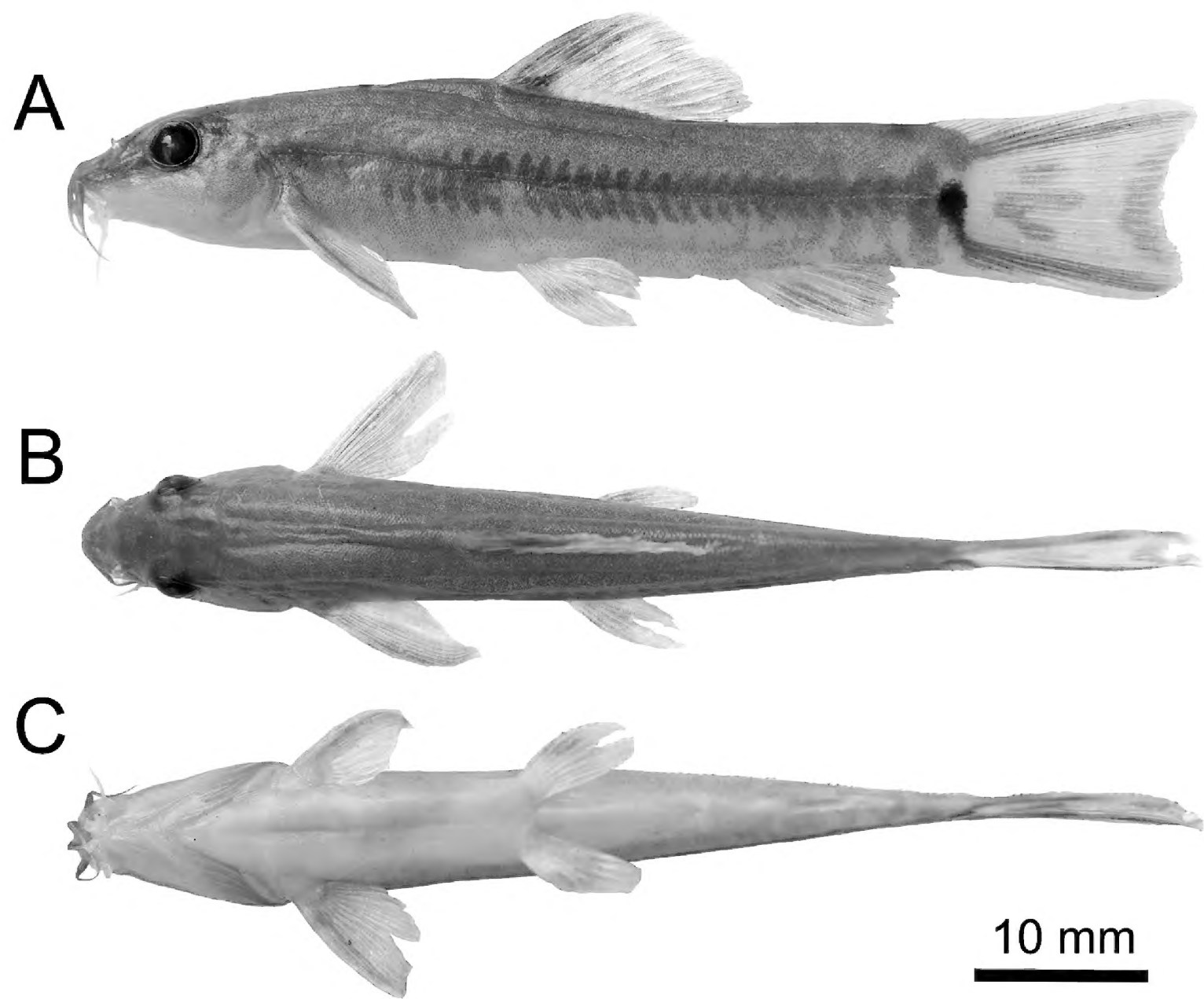
Data for *T. bacmeensis* are from Nguyen (2005) and Endruweit (2014), and *T. taeniatus* is from Freyhof and Serov (2001).

## Nomenclatural acts registration

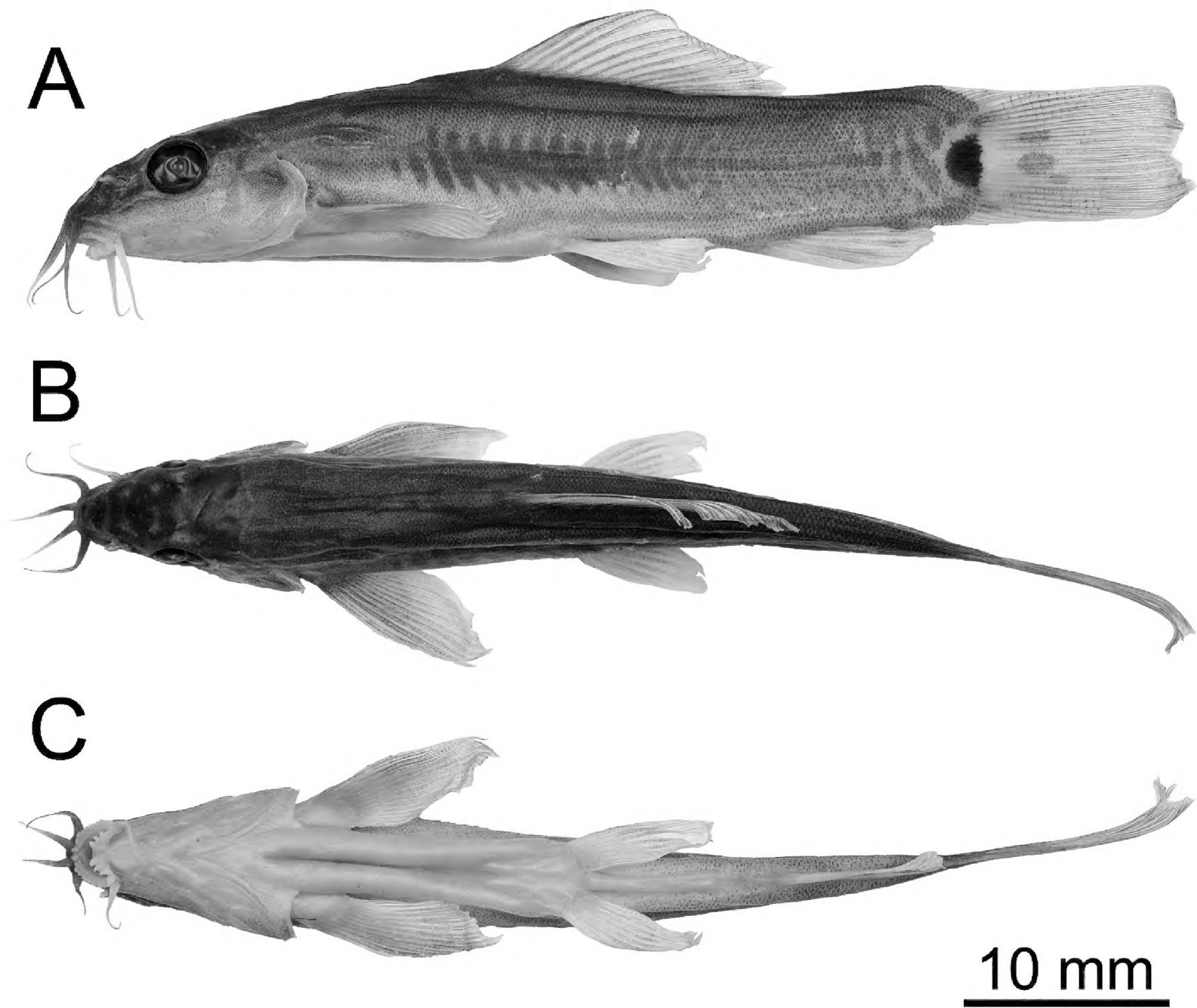
The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new name contained in the electronic version is effectively published under the Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank LSIDs (Life Science Identifiers) and can be resolved, and the associated information can be viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>.



**Figure 5.** Illustration of *T. pulcher* from the original description (Nichols and Pope 1927).



**Figure 6.** Lateral (a), dorsal (b), and ventral (c) views of *T. pulcher* from Baisha Li Autonomous County, Hainan Island, China. Scale bar: 10 mm.



**Figure 7.** Lateral (a), dorsal (b), and ventral (c) views of *T. zispi* from Nada Town, Hainan Island, China. Scale bar: 10 mm.



## Authors' Contributions

Z.-X.Q. measured the specimens, analyzed the data, and prepared the manuscript. F.L. and L.-N.D. conceived and designed the study and reviewed and edited the manuscript. J.-J.Z. participated in the observation of the new species specimens. All authors read and approved the final version of the manuscript.

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